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# Influence of *Crassostrea gigas* (Thunberg) sexual maturation stage and ploidy on uptake of paralytic phycotoxins

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## Abstract:

The purpose of this study was to assess paralytic phycotoxin uptake in diploid and triploid oysters at two stages of their sexual cycle corresponding to their status in early summer (June) and winter (November). Samples of diploid and triploid oysters were exposed to a toxic culture of *Alexandrium minutum* for 4 days in each season. No significant differences in filtration or clearance rates were observed during either November or June experiments. When diploid oysters were at resting stage (November), toxin uptake showed no significant difference between the ploidy classes. In contrast, when the diploid oysters were at the peak of their sexual maturation (June), the triploid oysters were seen to accumulate almost double the amount of paralytic toxins as the diploid ones.

**Keywords:** Paralytic shellfish poisoning ; *Crassostrea gigas* ; Diploid ; Triploid ; Contamination

## Report

1  
2 Since the 1970s, there has been a worldwide increase in the number of toxic events linked to  
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4 phycotoxins (Hallegraeff 1995, Bricelj & Shumway 1998a, 1998b). To date, based on their  
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6 effects upon human health, about twelve different families of toxic compounds synthesised  
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8 by marine microalgae have been discovered. The most hazardous phycotoxins found along  
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10 French coasts are paralytic shellfish toxins (PSTs). These were detected in France for the  
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12 first time in 1988 (Nezan and Ledoux, 1989; Lassus et al, 1994), in the „Abers“ area of  
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14 northern Brittany, where the causal micro-alga was soon identified as *Alexandrium minutum*.  
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16 Other French areas were also later found to be contaminated by toxigenic *Alexandrium* spp.  
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18 (Masselin et al. 2000, Sechet et al. 2003).  
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22 Phycotoxins are usually transferred from phytoplankton to man by marine vectors,  
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24 such as bivalve molluscs, herbivorous or carnivorous crustaceans, or fish.  
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27 Data from French National Committee of Shellfish Farming (CNC) illustrate that  
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29 shellfish farming is a common and important industry in Europe. Spain is in top position,  
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31 producing about 270,000 tonnes of shellfish per year, followed by France with 200,000  
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33 tonnes produced per year. For oyster farming, however, France is the largest European  
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35 producer with an annual average output of 128,500 tonnes (*Crassostrea gigas*). Nowadays,  
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37 as far as French shellfish farming is concerned, triploid oyster production represents a  
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39 significant proportion of the market. According to different estimations, triploids make up 30  
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41 to 50% of the total oyster production in France. Triploid oysters are attractive to producers for  
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43 two main reasons: firstly, in contrast to their diploid counterparts, the inhibition of  
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45 reproduction in triploid oysters means they can be sold throughout the year; secondly, some  
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47 past studies showed them to have lower summer mortality than diploids due to a reduced  
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49 energy expenditure (Kesarcodi-Watson et al. 2001, Garnier-Géré et al. 2002), a limited  
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51 protein metabolism (Hawkins and Day 1996) and a more efficient immune system (Nell 2002,  
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53 Duchemin et al. 2007). These last two characteristics provide them with an increased  
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55 resistance under stress conditions, particularly towards pathogens and contaminants.  
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2 To date, studies on the interactions between PST-producing phytoplankton and  
3 bivalve molluscs have mainly focused on toxin accumulation, biotransformation and  
4 detoxification mechanisms (Lassus et al. 2004, 2005; Guéguen et al. 2008). It also appears  
5 crucial that studies on the tendency of bivalves to accumulate toxins should consider all  
6 aspects of oyster physiology, including the reproductive process. Exposure to *Alexandrium*  
7 *spp.* is known to lead to various physiological disorders in oysters, including i) changes in *C.*  
8 *gigas* valve-opening behaviour when exposed to *Alexandrium minutum* (Lassus et al. 1999;  
9 Tran et al. 2010; Haberkorn et al. 2011.), ii) lesions in the mantle and gills (Nielsen and  
10 Stromgren, 1991; Haberkorn et al. 2010a), iii) inflammation of the digestive gland with  
11 massive hemocyte infiltration through diapedesis, and iv) myoatrophy with muscular  
12 degeneration and disruption of lipid synthesis (Haberkorn et al. 2010a and b).  
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29 Furthermore, the ANSES (the French Agency for Food, Environmental and  
30 Occupational Health & Safety), in a memo published in 2001 (N: 2001-SA-0080),  
31 recommended the implementation of a research programme to investigate and compare  
32 bioaccumulation and detoxification in triploid and diploid oysters when exposed to either  
33 heavy metals, bacteria or phycotoxins. At that time no data were available on phycotoxins  
34 and, therefore, it appeared interesting to compare paralytic toxin bioaccumulation yields in  
35 diploid versus triploid oysters.  
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45 Although no specific research has been done on the impact of reproductive period on  
46 PST uptake and accumulation in oyster soft tissues, recent studies (Haberkorn et al. 2010b)  
47 have reported differences in overall bioaccumulation between reproductively mature diploid  
48 and triploid oysters (which have inhibited gametogenesis). However, these authors did not  
49 consider daily bioaccumulation rates, toxin profile changes or filtration rates.  
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57 So, given the lack of detailed published research on the impact of reproductive stage,  
58 it was important to compare the role of filtration rates, ploidy and toxin profiles on toxin  
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1 uptake when oysters were exposed to PST-producing algae either during their reproductive  
2 resting stage and at the peak of their sexual maturity.  
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5 In a laboratory experiment, we took diploid and triploid oysters that were initially free  
6 from all contamination and whose ploidy had been individually confirmed by flow cytometry,  
7 and exposed sets of them to toxic algal cells at two stages of the diploid sexual cycle: in early  
8 winter (resting phase) and in early summer (maximum sexual maturity). The toxic  
9 dinoflagellate strain used for this contamination was *Alexandrium minutum* (AM89BM), which  
10 was grown in L1 medium at  $16 \pm 1^\circ\text{C}$ , with  $50 \pm 4 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  illuminance and a  
11 12/12 hour photoperiod. In November, twenty adult-sized oysters were placed in individual  
12 850 mL trays (Lassus et al. 2007) at  $16.0 \pm 0.4^\circ\text{C}$ , with a continuous supply of seawater  
13 (pumping rate:  $150 \text{ ml mn}^{-1}$ ). The *A. minutum* concentration of this recirculated seawater was  
14 continuously adjusted to  $290 \pm 80 \text{ cells mL}^{-1}$  and, as the mean toxicity of this alga was  $1.15$   
15  $\pm 0.07 \text{ pg equiv. STX cell}^{-1}$ , this corresponded to a calculated toxic intake of  $333.5 \pm 110.4 \text{ pg}$   
16  $\text{mL}^{-1}$ . In June, mean cellular toxicity was lower, i.e  $0.38 \pm 0.06 \text{ pg equiv. STX cell}^{-1}$  and  
17 therefore contamination of oysters was carried out with a higher continuous supply of  $520 \pm$   
18  $140 \text{ cells mL}^{-1}$  *A. minutum* corresponding to a toxic intake equal to  $206 \pm 84.4 \text{ pg mL}^{-1}$ . The  
19 contamination was continued over 4 days, during which biodeposits (faeces and pseudo-  
20 faeces) were collected every day, making it possible to calculate filtration and clearance  
21 rates and thus compare oyster feeding behaviour using Hawkins et al. (1996) formulae.  
22 Finally, toxin content in oysters was quantified by HPLC-FLD using the method of Oshima  
23 (1995), as slightly modified by Masselin et al (2001) and toxicity was calculated from  
24 Oshima's conversion factors. Gonyautoxins GTX1, GTX2, GTX3, GTX4, dc-GTX3 and dc-  
25 GTX2, were directly separated whereas C1 and C2 toxins were detected indirectly and  
26 quantified following acidic hydrolysis of samples.  
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54 Toxicities expressed as  $\mu\text{g STX eq. } 100 \text{ g}^{-1}$  shellfish flesh, and relative amounts of STX  
55 analogues were compared between diploid and triploid oysters within each experiment. No  
56 significant difference was observed between toxin contents of diploids and triploids at the  
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1 resting stage (ANOVA,  $p > 0.05$ ; Fig. 1A). In contrast, when contamination was performed  
2 when diploid oysters were at the peak of sexual maturation, the triploid oysters were  
3 significantly more contaminated than their diploid counterparts on days 2 (ANOVA,  $p < 0.05$ ),  
4 3 (ANOVA,  $p < 0.05$ ) and 4 (ANOVA,  $p < 0.01$ ; Fig. 1B). For clearance (Fig. 2A and 2B) and  
5 filtration (Fig. 2C and 2D) rates, and toxin profiles, diploid and triploid oysters showed no  
6 significant differences whatever the reproductive stage considered (Fig. 2E shows the  
7 example of the peak of sexual maturation)  
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10 The significant differences in toxin uptake detected during the June experiment (Fig.1B)  
11 could be explained by the difference in the physiological state of the diploid oysters  
12 compared with the November experiment. In June, triploid oysters have a more active  
13 metabolism (especially oxidative metabolism) than diploid ones (Soletchnik et al. 1996),  
14 which are in late gametogenesis. Alternatively, dissimilarity in sensitivity or tolerance to PSTs  
15 could also explain the discrepancies observed in the amounts of PSTs accumulated by  
16 diploid and triploid oysters exposed to *A. minutum*. In a similar way, Haberkorn (2009) and  
17 Haberkorn et al. (2010b) found about twice the amount of toxins in triploid oysters as in  
18 diploid oysters in April, May and June (experiments repeated four times). In these studies, on  
19 the basis of different physiological parameters (gonad maturation state, phospholipid levels  
20 and amylase activity in the digestive gland, plasma and haemocytes specific parameters),  
21 the authors hypothesised that the difference in toxicity observed is the result of a difference  
22 in feeding or metabolic activity (Haberkorn 2009, Haberkorn et al. 2010b). Our observations  
23 did not reveal any differences in the filtration or clearance rates. The dissimilarity in toxicity  
24 could be due to a difference either in the assimilation rate or metabolism. However, no  
25 difference was observed in November. It is also important to note that metabolic and feeding  
26 responses in the Pacific oyster *Crassostrea gigas* vary according to sexual maturation stages  
27 (Soletchnik et al. 1996), although, in our case, no significant difference in clearance rate was  
28 observed in relation to sexual stage. However, according to Soletchnik et al. (1996), the  
29 oyster energy balance at the end of maturation is negative ( $-15 \text{ J h}^{-1}$ ) while it remains  
30 between 110 and 170  $\text{J h}^{-1}$  at the immature stage or for post-spawning oysters. This energy  
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1 deficit observed at the end of sexual maturation could be due to a decrease in the absorption  
2 function. Diploid oysters would thus have a weaker absorption rate than triploid oysters at the  
3 end of sexual maturation. In a similar way, Berthollet-Marolle (2007) observed a better  
4 absorption rate in triploid oysters after April. Moreover, the lower mortality of triploid oysters  
5 can be explained by their low energy expenditure (notably for respiration and excretion)  
6 compared with diploid oysters, which confers an increased resistance to stress conditions  
7 (Kesarcodi-Watson et al. 2001b; Garnier-Géré et al. 2002). This low energy expenditure is  
8 due to a limited metabolism of proteins (Hawkins and Day, 1996). We can thus hypothesise  
9 that the contamination of diploid oysters is less than that of triploid oysters as a result of a  
10 change in the metabolism of diploid oysters at the sexual maturation stage.  
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22 It would be interesting to carry out further investigations on the influence of ploidy on  
23 detoxification, as recommended by ANSES. Furthermore, the present study has suggested  
24 that there is a possible effect of the reproductive status on PST uptake in diploid oysters. It  
25 would thus be worth testing the influence of oyster physiological status on toxin accumulation  
26 by repeating the experiments of the present study, under the same conditions, but at several  
27 more stages of reproduction.  
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## FIGURES

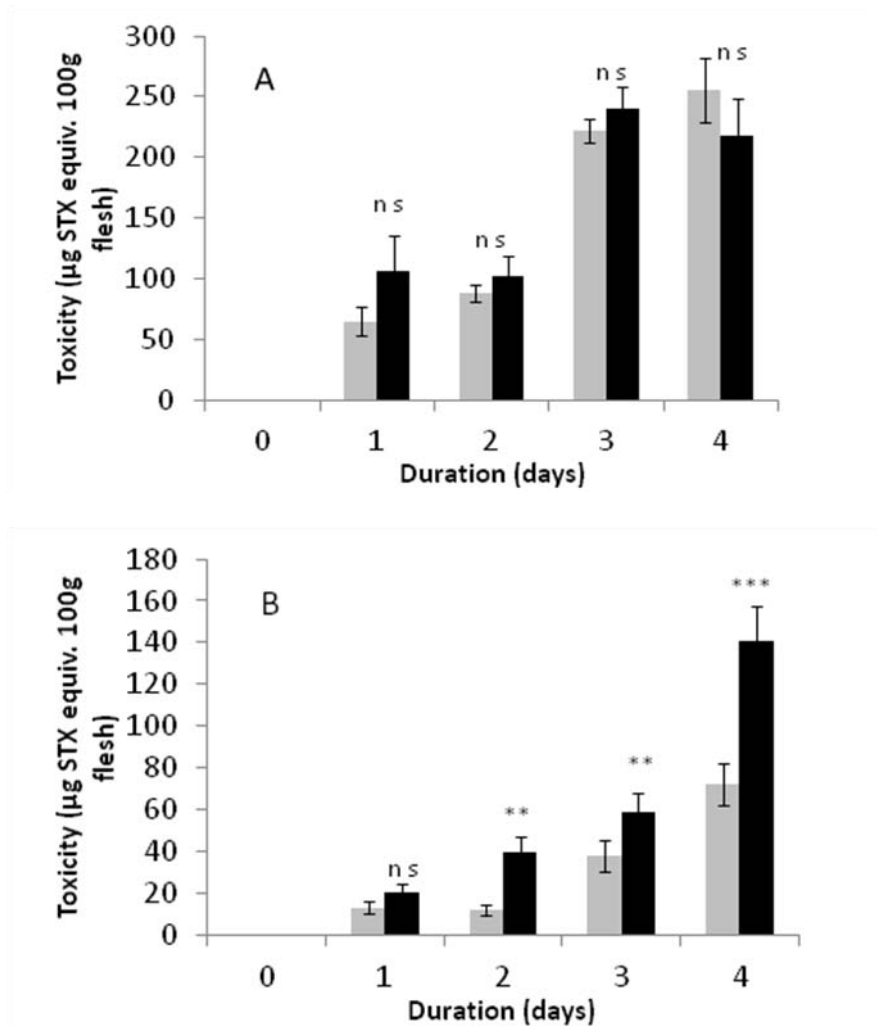


Figure 1: Toxin content of diploid (grey) and triploid (black) oysters over 4 days of contamination by *Alexandrium minutum* in November (A), at sexual resting stage for diploid oysters, and in June (B), at the peak of sexual maturation for diploid oysters. Mean  $\pm$  s.d.; ns: not significant, significant differences labelled \*\* :  $p < 0.05$  or \*\*\* :  $p < 0.01$

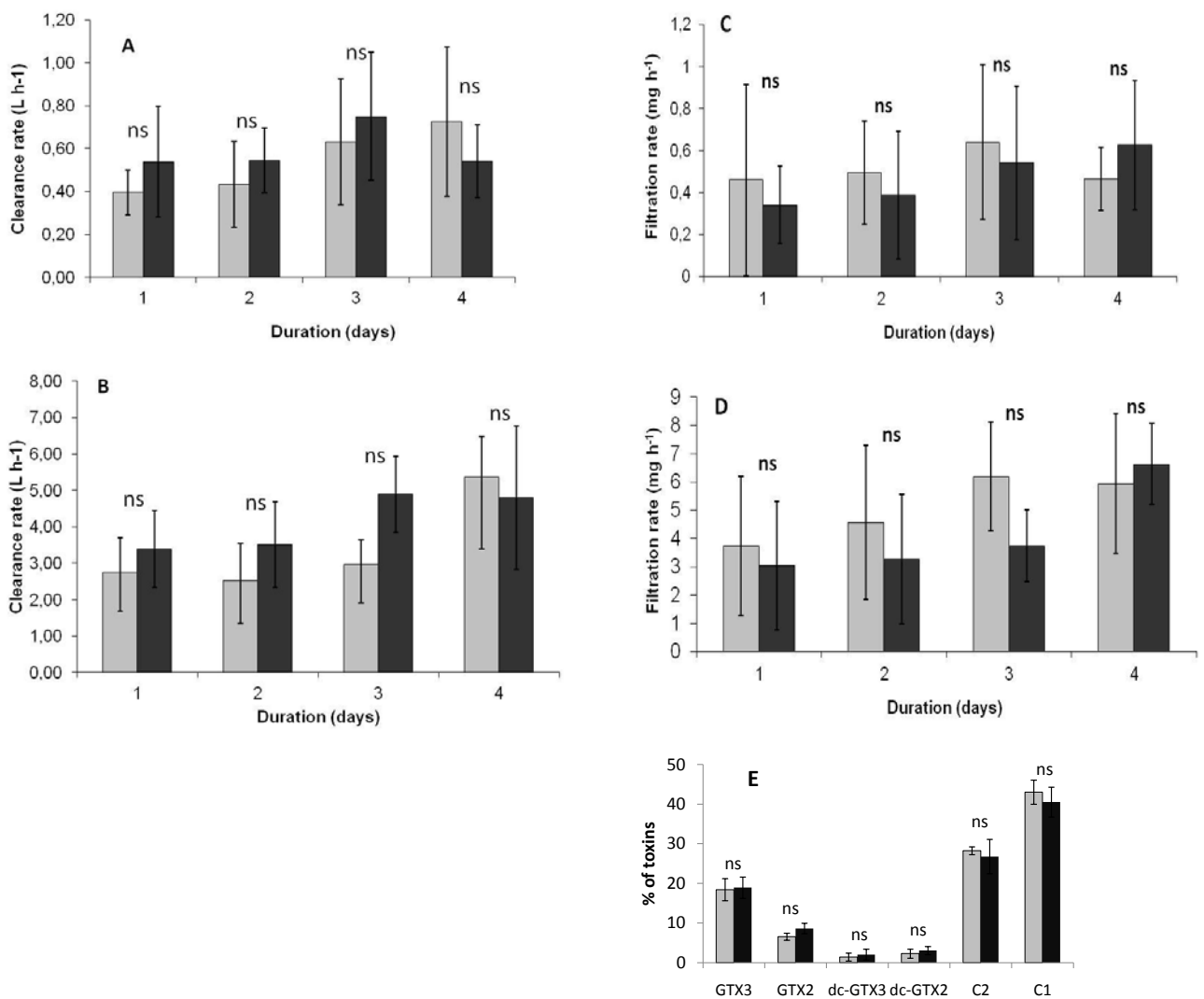


Fig 2 : Evolution of clearance rate (A and B) and filtration rate (C and D) over 4 days contamination of diploid (grey) and triploid (black) oysters by *Alexandrium minutum*. In A and C, the diploid oysters were at resting stage; in B and D, they were at the end of sexual maturation. E shows the toxin profile for day 4 of the experiment done at the peak of diploid sexual maturity. Means  $\pm$  s.d.; ns: not significant. GTX: gonyautoxins, dc-: decarbamoyls, C: C toxins